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Potentials of essential oils of *Chenopodium ambrosioides* L. and *Cupressus sempervirens* L. against stored maize pest, *Sitophilus zeamais* Motschulsky

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Abstract
Maize is cultivated worldwide and used as food for man and animals as well as in fuel production is usually attacked and destroyed during storage by *Sitophilus zeamais.* With inaccessibility to the highly toxic non-biodegradable synthetic pesticides, poor peasant farmers are left just with the choice of locally available less toxic plants as pesticide alternatives. It is with this in mind that we tested the insecticidal potentials of essential oils of *Chenopodium ambrosioides* and *Cupressus sempervirens* against *S. zeamais* on stored maize. Mortality, progeny inhibition, repellence and persistence were tested. Pesticidal abilities of both oils increased with dose of application. 200 µL/kg of *Ch. ambrosioides* caused 100% mortality in 72 hours as well as completely inhibited progeny production. 8µL of both oils were repellent to the weevils. The persistence of both oils however dropped to zero within two weeks. Therefore both plants stand recommended as low persistence botanicals against *S. zeamais.*

Keywords: Botanical, essential oil, persistence, maize insect pests

Introduction

Food security and poverty alleviation is primordial for any country. Sub-Saharan Africa is the most vulnerable region with the average amount of food available per person per day in the region being 1,300 calories compared to the world wide average of 2,700 calories. Food security crisis in the Sahel, driven by chronic poverty, malnutrition, high food prices, drought and low agricultural production, affected 18.7 million people across the region in 2013 [1]. Products from farming serve for on-farm consumption and generating income. Cereals are a major source of food and contributed to about 50% of the total dietary energy supplies (kcal) for this region for 2007-2009 [2]. Maize is the most widely-grown staple food crop in Sub-Sahara Africa (SSA) occupying more than 33 million ha each year. In 2012, it had the best yield in Africa (70,076,591 tons) followed by sorghum, wheat and rice [3]. It is a major staple food crop grown in diverse agro-ecological zones and farming systems, and consumed by people with varying food preferences and socio-economic backgrounds in SSA. Its central role as a staple food is comparable to that of rice or wheat in Asia, with consumption rates being the highest in eastern and southern Africa [4]. Cameroon is a country with the backbone of its economy being agriculture. About 70% of its active population is involved in agriculture, which contributes to about 25% of the GDP [2], 55% of its population in the rural environment have agriculture as main activity [5] Most of these peasant farmers however live in extremely poor conditions and their practice of agriculture is rendered difficult by the absence of farming tools, fertilizers, education, farm to market roads, drying and storage facilities and pest problems [6]. Therefore, plants which make excellent leads for new pesticide development [7] could be used. *Chenopodium ambrosioides* L. (Amaranthaceae) and *Cupressus sempervirens* L. (Cupressaceae) widely used as plants for insecticidal purposes were chosen for this work. *Ch. ambrosioides* L. (Amaranthaceae) is a plant whose powders have been studied against *Sitophilus zeamais* Motschulsky for Toxicity, oviposition suppression, ovicidal and larvicidal effects [8, 9, 10]. Tapondjou *et al.* [10] did *in-vitro* toxicity and progeny control effects using the essential oils while with *Cu. sempervirens* L. (Cupressaceae), mortality, progeny and Repellency effects have also been studied [11, 12] on *S. zeamais* Motschulsky.

The main objective of this work was the use of essential oils of *Ch. ambrosioides* L. and *Cu. sempervirens* L. available locally to control the destruction of stored maize by *S. zeamais* Motschulsky. And specifically to evaluate their effectiveness on insect: Mortality, progeny production, repellence, persistence as pesticides *in-vivo* on treated Acid Tolerant Population (ATP) maize grains. This is an alternative response to the use of synthetic pesticides that are not easily biodegradable, are biotoxic, promote the development of resistance in insects and are not usually available.

Materials and methods

a) Plant Material

a.1) Test maize

The Acid Tolerant Population (ATP) variety of maize was collected from farmers in Big Babanki (North West Region, Cameroon) and identified in the cereals unit of IRAD (Institute of Agricultural Research for Development) Bambui. The water content of maize used in bioassays was evaluated. ANFOR method and found to be 12.669%. The weevils were gotten from stock cultures from the crop protection laboratory of the Institute of Agricultural research for Development (IRAD, Bambui)

Fresh leaves of *Ch. ambrosioides* and *Cu. sempervirens* were collected from the experimental field of IRAD Bambui during December 2015 - February 2016, shade dried, and hand crushed to get powder.

a.2) Extraction of Essential Oils by Hydrodistillation

Essential oils were extracted by hydrodistillation (water distillation) with the help of a Clevenger apparatus at the Laboratory of Industrial Chemistry and Bio-resources of the National Advanced School of Agro-Industrial Sciences (ENSAI, Ngaoundere). The oils collected were dried in $Na_2SO_{4(s)}$ column, weighed and stored at 4 ºC in opaque bottles in the absence of direct light. All bioassays were carried out from May to September 2015.

a.3) Mortality and progeny control

25 g of maize was placed in 500 mL glass jars. Aliquots of the *Ch. ambrosioides* and *Cu. sempervirens* were applied at the following concentrations 0 µL (control), 5 µL, 10 µL; 15 µL, 20 µL (all diluted in 1mL acetone). All treatments were replicated 4 times. The maize-essential oil-acetone mixture was then manually shaken to permit complete coating of the maize by the essential oils. After manual shaking, the jars were left open for 45 minutes on laboratory shelves to permit complete evaporation of the solvent. Afterwards, 20 unsexed adults (less than 7 days old) were separately added into each jar and kept on laboratory shelves. Insect mortalities were recorded 1, 3, 7 and 14 days post treatment and percentage insect mortality was corrected using the Abbott [13] formula. All tests were carried out at the following conditions: temperature: 17.3–28.8°C and relative humidity: 56.3–97.8%

F1 progeny assessment

On the 14th day post-infestation, the remaining live insects were removed and the different jars containing grains were kept under the same experimental conditions. The recording of F1 progeny was done once a week for 5 weeks commencing 6weeks post-infestation [14].

Percentage reduction in adult emergence or inhibition rate (% IR) was calculated as:

$$\%IR = \frac{(Cn - Tn)x100}{Cn}$$

Where C_n is the number of newly emerged insects in the untreated (Control) jar and T_n is the number of insects in the treated jar

a.4) Repellency test

The repellence test used was adopted from several authors. [15] [16]. Four solutions of 1 µL, 2 µL; 4 µL, 8 µL of essential oils were prepared by each dissolving essential oils in 0.5 ml acetone. Whatman nº1 filter papers were cut into two equal halves one half of each dish (110 mm diameter) was treated with essential oil solution as uniform as possible by using micro pipette. The other half of the filter paper was treated with acetone only. The essential oil treated and acetone treated filter papers halves were air-dried to evaporate the solvent completely. Essential oil treated and acetone treated half-dishes were then attached lengthwise, edge-to-edge with adhesive tape and placed at the bottom in glass petri dish (height 15 mm × radius 55 mm). Ten adults of insects were released at the centre of the petri dishes and then petri dishes were covered and kept in dark. Four replicates were set for each concentration of essential oils. Number of the insects on both treated and untreated halves was recorded every hour for four hours in mild light. The average was then calculated. The percentage repellence was calculated using the formula by Talukder & Howse [16] given by PR=2 X (C-50) where: C is the percentage of insects in the negative control half. The results were interpreted following the scale by McDonald *et al*. [15]

a.5) Persistence bioassay

50 g seeds each treated with 40 µL EO/2mL acetone as in the mortality bioassay were infested by a group of 20 insects after 1 day, 1 week 2 and 4 weeks post infestation. At each persistence time assessment, the experiment was similar to that of the toxicity bioassay.

a.6) Statistical analysis

Adult mortality was corrected relative to natural mortality in the controls using Abbott (13) formula. Data on mortality and progeny production was transformed by using $\sqrt{(x + 0.5)}$, then later ANOVA was done using statistical package for social sciences (SPSS) software. Tukey test (HSD) was used for mean separation. Probit analysis [17] was used for the lethal doses that cause 50% (LD_{50}) and 90% (LD_{90}) mortality after 1, 3, 7 and 14 days after treatment.

Results and discussion

a) Mortality

The mortality of *Sitophilus zeamais* by contact upon treatment with essential oils of *Ch. ambrosioides* and *Cu. sempervirens* are shown in Table 1. Mortality generally increased very significantly with dose administered and period of exposure. There were also significant differences between the same concentrations of essential oils of both plants in the different periods of exposure. The highest dose of *C. ambrosioides* killed over 90% of the weevils after 24 hours of exposure against 54% with *Ch. sempervirens.* Tapondjou *et al.* [11] got 5% mortality from 0.2 mL/cm^2 of filter paper *in vitro* after 24h also with *S. zeamais* being the least susceptible of all the tested insects to *Ch. ambrosioides* essential oil. They also found *Eucalyptus saligna* to be more active than *Cu. sempervirens*. These mortalities had high significant differences between them. However the other doses had no significant differences in their mortalities after 24 hours of exposure. Similarly, Ntonifor *et al.* [9] working on powders of *Ch. ambrosioides* also got 100% mortality of *S. zeamais* at a dose of 20 g/kg.

Table 1: Mortality (Mean ± S.E) of *Sitophilus zeamais* due to treatment of maize seeds with essential oils of *Chenopodium ambrosioides* and *Cupressus sempervirens*

Exposure Period (days)	Content (µL/kg)	Mortality		*t*
		Ch. ambrosioides	***Cu. sempervirens***	
1	00	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	/
	25	11.25±1.25^{A}	8.75 ± 1.25^{A}	1.14^{ns}
	50	2.50 ±1.44^{B}	2.50 ± 1.25^{A}	0.00^{ns}
	100	31.25 ± 2.39^{C}	33.75 ± 2.39^{B}	-0.73^{ns}
	200	88.75 ± 2.39^{D}	50.00 ±4.56^{C}	7.51**
F		447.569***	79.319***	
LC_{50} (µL/kg)		10.35	8.95	
3	00	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	/
	25	12.5±2.89^{A}	13.75 ±2.39^{B}	-0.447^{ns}
	50	13.75 ±1.25^{B}	12.50± 1.44^{B}	-4.58**
	100	43.75 ±2.39^{C}	42.50 ±3.23^{C}	0.31^{ns}
	200	100± 0.00^{D}	53.75±2.39^{D}	19.32***
F		126.004***	106.402***	
LC_{50} (µL/kg)		10.54	14.77	
7	00	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	/
	25	36.25±2.39^{B}	26.25 ±2.39^{B}	2.95*
	50	31.25 ±2.39^{B}	33.75±2.39^{B}	-0.73^{ns}
	100	88.75±4.27^{C}	56.25 ±2.39^{C}	6.64**
	200	100± 0.00^{D}	68.75±62.50^{C}	5.0*
F		296.184**	63.861***	
LC_{50} (µL/kg)		36.72	31.30	
14	00	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	/
	25	34.54±2.93^{B}	38.36 ±2.82^{B}	-0.99^{ns}
	50	42.30±1.02^{B}	47.30± 3.17^{B}	-1.5^{ns}
	100	92.30±3.38^{C}	67.96 ±2.39^{C}	5.87**
	200	100± 0.00^{C}	81.97 ±3.44^{D}	5.24*
F		416.000*	138.822***	
LC_{50} (µL/kg)		41.09	43.06	

Means ± S.E. in the same column/row for the same category of insecticide, followed by the same letter do not differ significantly at $P = 0.05$ (Tukey's test). Each datum represents the mean of four replicates of 20 insects each. *: significant ($P<0.05$); **: very significant ($P<0.01$): ***: very highly significant ($P<0.001$).

By the 14th day both insecticides presented appreciable toxicity of more than 80%. Mortality generally increased with dose and period of exposure. 100% *Chenopodium* presented the most efficient insecticidal activity from day 1 to day 14 after infestation. In fact, mortality was at 100% after 72 hours of treatment. Tapondjou *et al.* [11] also got 100% mortality of *S. zeamais* after 48 hours *in-vitro* by treating with *Ch. ambrosioides* at a dose of 6.4%. This is in agreement with our results which showed that 0.02% ($2*10^{-3}$L/Kg) caused 100% mortality after 72 hours. The toxicity of the volatile oil from *Ch. ambrosioides* is generally attributed to ascaridole, cymol and a-terpinene (Malloy, 1923; Pollacketal, 1990 cited by Tapondjou *et al.,*) [10]. The toxicity of *Ch. ambrosioides* on *S. zeamais* was also shown by Abiodoun *et al.* [18] both as contact powder and as fumigant ethanolic extract/ essential oil causing 100% mortality in 48 hours with appreciable LC_{50}.

b) Progeny Control

From table 2, it is noticed that progeny emergence control was also dose dependent. The highest doses of both essential oils gave percentage inhibitions in progeny greater than 90 percent relative to the control. There were very high significant differences in the percentage reduction in progeny production between all the doses administered for both plants.

Table 2: F1 progeny (Mean ± S.E) of *Sitophilus zeamais* due to treatment of ATP maize seeds with essential oils of *Chenopodium ambrosioides* and *Cupressus sempervirens*

Product	*Ch. ambrosioides*		*Cu. sempervirens*	
Content (µL/kg)	***F1***	**% reduction of *F1***	***F1***	**% reduction of *F1***
00	42.75±1.25^{D}	0.00 ± 0.00^{A}	42.75 ±1.25^{E}	0.00 ± 0.00^{A}
25	11.75±0.48^{C}	72.39±1.07^{B}	21.75±0.85^{D}	49.08±1.79^{B}
50	6.25±0.85^{B}	85.36±2.01^{C}	14.25±0.63^{C}	66.60±1.62^{C}
100	3.25±0.48^{B}	92.45±1.00^{D}	9.25±0.48^{B}	78.31±1.31^{D}
200	0.00 ± 0.00^{A}	100.00±00^{E}	3.00 ±0.82^{A}	92.98±0.95^{E}
F	543.659***	1032.575***	381.912***	759.928***

Means ± S.E. in the same column for the same category of insecticide, followed by the same letter do not differ significantly at $P = 0.05$ (Tukey's test). Each datum represents the mean of four replicates of 20 insects each. ***: very highly significant ($P<0.001$)

All products were good progeny production inhibitors with very high significant differences. Chenopodium gave 100% progeny production inhibition at its highest dose**.** These results are in agreement with those of Tapondjou *et al.* [11] who also found good progeny control properties of *Cu. sempervirens.* Appreciable ovicidal and lavicidal effects of powders of *Ch. ambrosioides* and *Cu. sempervirens* against *S. zeamais* have also been proven [11, 9]. This progeny control properties of the essential oils are a direct indication of content both in the powders and essential oils of these plants, chemicals that control progeny production.

c) Repellence

Table 3 shows the trend of repellence of the various products. Generally, the 8 µL/kg dose was the most repellent of all the products with repellence indices of more than 60% each. *Ch. ambrosioides* however was more repellent than *Cu. sempervirens* in all the doses administered. The repellence of *Cu. sempervirens* was earlier proven to even be greater than that of pure cymol [11]

Table 3: *In-vitro* repellency (Mean ± S.E) of *Sitophilus zeamais* on filter paper due to treatment with essential oils of *Chenopodium ambrosioides* and *Cupressus sempervirens*

Product	Content (µL/kg)	Percentage Repellence	Class	Interpretation
Ch. ambrosioides	0	0.00 ± 0.00^{A}	/	/
	1	5.00±5.00^{AB}	I	Very low repellence
	2	32.50±7.50^{BC}	II	Low repellence
	4	50.00±5.77^{CD}	III	Moderately repellent
	8	75.00±9.57^{D}	IV	Repellent
	F	23.788**		
Cu. sempervirens	0	0.00 ± 0.00^{A}	/	/
	1	15.00±5.00^{AB}	II	Low repellence
	2	30.00±5.78^{ABC}	II	Low repellence
	4	35.00±9.57^{BC}	III	Moderately Repellent
	8	55.00±9.50^{C}	IV	Repellent
	F	8.948**		

Means ± S.E. in the same column for the same category of insecticide, followed by the same letter do not differ significantly at P = 0.05 (Tukey's test). Each datum represents the mean of four replicates of 10 insects each. **: very significant (P<0.01)

Persistence

The persistence of an essential oil reduces quickly with time due to its highly volatile nature. Therefore, as duration post treatment increases, the essential oil evaporates leaving the product without significant protection. The decrease in persistence with duration is presented in Table 4. This decrease however is faster than that of the powder. Ntonifor *et al.* [9] found that the persistence of powders took up to 3 weeks to drop to zero. This could also be explained by the fact that apart from the fumigant effect of the powder chemical components, the powder particles also exert additional insecticidal potentials on the insects.

Table 4: Persistence (Mean ± S.E) of essential oils of *Chenopodium ambrosioides* and *Cupressus sempervirens* on treated ATP maize seeds infested with *Sitophilus zeamais*

Period post treatment (days)	Mortality		*t*
	Ch. ambrosioides	*Cu. sempervirens*	
0	100± 0.00^{C}	81.97 ±3.44^{D}	5.24*
1	27.63±1.32^{C}	21.25 ±1.25^{C}	2.910^{ns}
7	11.75±1.18^{B}	10.00±2.04^{B}	0.646^{ns}
14	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	/
F	2577.337***	318.133***	

Means ± S.E. in the same column/row for the same category of insecticide, followed by the same letter do not differ significantly at P = 0.05 (Tukey's test). Each datum represents the mean of four replicates of 20 insects each. ^{ns} non-significant, *: significant (P<0.05) ; ***: very highly significant (P<0.001).

Persistence reduced very significantly from zero hour post treatment to fall to zero mortality after two weeks in all the products evaluated. At zero days, *Chenopodium* was the most efficient (100% mortality). We also noticed high significant differences in the persistence of both products in at the f treatment. However, as duration increased these significant differences disappeared and the different persistence results had no significant differences. In both products, there were very high significant differences between all the persistence periods. The toxicity of the ethanol extract was lost after 24 h of treating the seeds with either ethanol extract or essential oil of *Ch. ambrosioides* as indicated by sharp increases in LC_{50} values [8]. Since the present study showed that the bioactivity of *C. ambrosioides* against test insects was non-persistent. Such results indicate the need for repeated applications in order to provide effective protection of stored grains when *Ch. ambrosioides* products are used as protectants. On the other hand, if repeated treatments are not done, once treatment is done, the stored maize should be well protected from secondary infestation. The low persistence could also be considered a good factor for selection as good insecticide since problems of modification of taste, colour and odour of the stored product will be reduced.

Conclusion

Essential oils of *Ch. ambrosioides* and *Cu. sempervirens* available locally were tested *in vivo* on ATP maize variety. Both showed high insecticidal efficacy in all the bioassays except for persistence. The ability to control the proliferation of *S. zeamais* in stored maize was also dose dependent and increased with period of exposure to the insecticide. Therefore both insecticides stand highly recommended due to their insecticidal, progeny control effects, high repellence and low persistence.

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